

ABSTRACT

The present invention relates to the use of site-specific
5 nucleic acid nicking enzymes to create single-stranded regions
in duplex nucleic acids. Such single-stranded regions can take
the form of gaps interior to the duplex, or terminal single-
stranded regions. Single-stranded termini can be crafted to
allow linkage of various elements *via* base-pairing with
10 elements containing a complementary single-stranded region.
This joining is useful, for example, in an ordered, oriented
assembly of DNA modules to create cloning or expression
vectors. This joining is also useful in attaching detection
probes and purifying DNA molecules containing the single-
stranded region. Gaps are useful in similar applications,
15 including attaching detection or purification probes.